

WE CLAIM:

1. A method of identifying nucleic acid ligands to TGF β , comprising:
 - a) contacting a candidate mixture of nucleic acids with TGF β ,
wherein nucleic acids having an increased affinity to TGF β relative to the
5 candidate mixture may be partitioned from the remainder of the candidate
mixture; and
 - b) partitioning the increased affinity nucleic acids from the
remainder of the candidate mixture; and
 - c) amplifying the increased affinity nucleic acids to yield a mixture
10 of nucleic acids enriched for nucleic acid sequences with relatively higher affinity
and specificity for binding to TGF β , whereby nucleic acid ligands of TGF β may
be identified.
2. The method of claim 1 further comprising:
 - (d) repeating steps a), b), and c).
- 15 3. The method of claim 1 wherein said candidate mixture of nucleic acids
is comprised of single stranded nucleic acids.
4. The method of claim 3 wherein said single stranded nucleic acids are
ribonucleic acids.
5. The method of claim 4 wherein said nucleic acids comprise modified
20 nucleic acids.
6. The method of claim 5 wherein said nucleic acids are 2'-amino (2'-NH₂)
modified ribonucleic acids.
7. The method of claim 5 wherein said nucleic acids are 2'-F modified
ribonucleic acids.
- 25 8. The method of claim 5 wherein said nucleic acids are 2'-NH₂-UTP, 2'-
F-CTP modified ribonucleic acids.
9. The method of claim 5 wherein said nucleic acids are 2'-F-UTP, 2'-
NH₂-CTP modified ribonucleic acids.
- 30 10. The method of claim 3 wherein said single stranded nucleic acids are
deoxyribonucleic acids.

11. A method for treating TGF β -mediated pathological conditions comprising administering a pharmaceutically effective amount of a TGF β nucleic acid ligand.

12. The method of claim 11 wherein said TGF β nucleic acid ligand is identified according to the method of claim 1.

13. A method for treating TGF β 1-mediated pathological conditions comprising administering a pharmaceutically effective amount of a TGF β 1 nucleic acid ligand.

14. The method of claim 13 wherein said TGF β 1 nucleic acid ligand is identified according to the method of claim 1.

15. The method of claim 14 wherein said ligand is selected from the ligands of Table 3 and 6 (SEQ ID NOS:12-42; 55-89).

16. A purified and isolated non-naturally occurring nucleic acid ligand to TGF β .

17. A purified and isolated non-naturally occurring nucleic acid ligand to TGF β 1.

18. The purified and isolated non-naturally occurring nucleic acid ligand of claim 17 wherein said nucleic acid ligand is single-stranded.

19. The purified and isolated non-naturally occurring ligand of claim 18 wherein said nucleic acid ligand is ribonucleic acid.

20. The purified and isolated non-naturally occurring ligand of claim 18 wherein said nucleic acid ligand is deoxyribonucleic acid.

21. A nucleic acid ligand to TGF β identified according to the method comprising:

a) contacting a candidate mixture of nucleic acids with TGF β , wherein nucleic acids having an increased affinity to TGF β relative to the candidate mixture may be partitioned from the remainder of the candidate mixture; and

b) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture; and

c) amplifying the increased affinity nucleic acids to yield a mixture of nucleic acids enriched for nucleic acid sequences with relatively higher affinity and specificity for binding to TGF β 1, whereby nucleic acid ligands of TGF β 1 may be identified.

5 22. A nucleic acid ligand to TGF β 1 identified according to the method comprising:

a) contacting a candidate mixture of nucleic acids with TGF β 1, wherein nucleic acids having an increased affinity to TGF β 1 relative to the candidate mixture may be partitioned from the remainder of the candidate
10 mixture; and

b) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture; and

c) amplifying the increased affinity nucleic acids to yield a mixture of nucleic acids enriched for nucleic acid sequences with relatively higher affinity and specificity for binding to TGF β 1, whereby nucleic acid ligands of TGF β 1
15 may be identified.

23. The purified and isolated non-naturally occurring ribonucleic acid ligand to TGF β 1 of claim 19 wherein said ligand is selected from the group consisting of the sequences set forth in Table 3 (SEQ ID NOS:12-42).

20 24. The purified and isolated non-naturally occurring ribonucleic acid ligand to TGF β 1 of claim 19 wherein said ligand is substantially homologous to and has substantially the same ability to bind TGF β 1 as a ligand selected from the group consisting of the sequences set forth in Table 3 (SEQ ID NOS:12-42).

25 25. The purified and isolated non-naturally occurring ribonucleic acid ligand to TGF β 1 of claim 19 wherein said ligand has substantially the same structure and substantially the same ability to bind TGF β 1 as a ligand selected from the group consisting of the sequences set forth in Table 3 (SEQ ID NOS:12-42).

26. The purified and isolated non-naturally occurring deoxyribonucleic acid ligand to TGF β 1 of claim 20 wherein said ligand is selected from the group consisting of the sequences set forth in Table 6 (SEQ ID NOS:55-89).

5 27. The purified and isolated non-naturally occurring deoxyribonucleic acid ligand to TGF β 1 of claim 20 wherein said ligand is substantially homologous to and has substantially the same ability to bind TGF β 1 as a ligand selected from the group consisting of the sequences set forth in Table 6 (SEQ ID NOS:55-89).

10 28. The purified and isolated non-naturally occurring deoxyribonucleic acid ligand to TGF β 1 of claim 20 wherein said ligand has substantially the same structure and substantially the same ability to bind TGF β 1 as a ligand selected from the group consisting of the sequences set forth in Table 6 (SEQ ID NOS:55-89).

29. A method of identifying nucleic acid ligands of PDGF, comprising:
15 a) contacting a candidate mixture of nucleic acids with PDGF, wherein nucleic acids having an increased affinity to PDGF relative to the candidate mixture may be partitioned from the remainder of the candidate mixture; and
b) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture; and
20 c) amplifying the increased affinity nucleic acids to yield a mixture of nucleic acids enriched for nucleic acid sequences with relatively higher affinity and specificity for binding to PDGF, whereby nucleic acid ligands of PDGF may be identified.

30. The method of claim 29 further comprising:
25 d) repeating steps a) b), and c).

31. The method of claim 29 wherein said candidate mixture of nucleic acids is comprised of single stranded nucleic acids.

32. The method of claim 31 wherein said single stranded nucleic acids are ribonucleic acids.

33. The method of claim 31 wherein said single stranded nucleic acids are deoxyribonucleic acids.

Sub A36 34. The method of claim 32 wherein said nucleic acids comprise modified nucleic acids.

5 35. The method of claim 34 wherein said nucleic acids are 2'-amino (2'-NH₂) modified ribonucleic acids.

36. The method of claim 34 wherein said nucleic acids are 2'-fluoro (2'-F) modified ribonucleic acids.

10 37. A method for treating a PDGF-mediated disease comprising administering a pharmaceutically effective amount of a nucleic acid ligand of PDGF.

38. The method of claim 37 wherein said nucleic acid ligand is identified according to the method of claim 29.

15 39. The method of claim 38 wherein said nucleic acid ligand is selected from one of the ligands of Tables 8 and 13, and Figures 3, 4, and 10 (SEQ ID NOS.93-124, 128-176).

40. A purified and isolated non-naturally occurring nucleic acid ligand to PDGF.

20 41. The purified and isolated non-naturally occurring nucleic acid ligand of claim 40 wherein said nucleic acid ligand is single-stranded.

42. The purified and isolated non-naturally occurring nucleic acid ligand of claim 41 wherein said nucleic acid ligand is ribonucleic acid.

43. The purified and isolated non-naturally occurring nucleic acid ligand of claim 41 wherein said nucleic acid ligand is deoxyribonucleic acid.

25 44. A nucleic acid ligand to PDGF identified according to the method comprising:

30 a) contacting a candidate mixture of nucleic acids with PDGF, wherein nucleic acids having an increased affinity to PDGF relative to the candidate mixture may be partitioned from the remainder of the candidate mixture; and

b) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture; and

c) amplifying the increased affinity nucleic acids to yield a mixture of nucleic acids enriched for nucleic acid sequences with relatively higher affinity and specificity for binding to PDGF, whereby nucleic acid ligands of PDGF may be identified.

45. The purified and isolated non-naturally occurring ribonucleic acid ligand to PDGF of claim 42 wherein said ligand is selected from the group consisting of the sequences set forth in Table 13 (SEQ ID NOS:128-170).

46. The purified and isolated non-naturally occurring ribonucleic acid ligand to PDGF of claim 42 wherein said ligand is substantially homologous to and has substantially the same ability to bind PDGF as a ligand selected from the group consisting of the sequences set forth in Table 13 (SEQ ID NOS:128-170).

47. The purified and isolated non-naturally occurring ribonucleic acid ligand to PDGF of claim 42 wherein said ligand has substantially the same structure and substantially the same ability to bind PDGF as a ligand selected from the group consisting of the sequences set forth in Table 13 (SEQ ID NOS:128-170).

48. The purified and isolated non-naturally occurring deoxyribonucleic acid ligand to PDGF of claim 43 wherein said ligand is selected from the group consisting of the sequences set forth in Tables 8 and 9, and Figures 3, 4, and 10 (SEQ ID NOS:93-124, 171-176).

49. The purified and isolated non-naturally occurring deoxyribonucleic acid ligand to PDGF of claim 43 wherein said ligand is substantially homologous to and has substantially the same ability to bind PDGF as a ligand selected from the group consisting of the sequences set forth in Tables 8 and 9, and Figures 3, 4 and 10 (SEQ ID NOS:93-124, 171-176).

50. The purified and isolated non-naturally occurring deoxyribonucleic acid ligand to PDGF of claim 43 wherein said ligand has substantially the same structure and substantially the same ability to bind PDGF as a ligand selected from

the group consisting of the sequences set forth in Tables 8 and 9, and Figures 3, 4 and 10 (SEQ ID NOS:93-124, 171-176).

51. The purified and isolated non-naturally occurring nucleic acid ligand to PDGF of claim 40 comprising the conserved structure shown in Figure 3 (SEQ ID NO:171).

52. A method of identifying nucleic acid ligands to hKGF, comprising:

a) contacting a candidate mixture of nucleic acids with hKGF, wherein nucleic acids having an increased affinity to hKGF relative to the candidate mixture may be partitioned from the remainder of the candidate mixture; and

b) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture; and

c) amplifying the increased affinity nucleic acids to yield a mixture of nucleic acids enriched for nucleic acid sequences with relatively higher affinity and specificity for binding to hKGF, whereby nucleic acid ligands of hKGF may be identified.

53. The method of claim 52 further comprising:

d) repeating steps a), b), and c).

54. The method of claim 52 wherein said candidate mixture of nucleic acids is comprised of single stranded nucleic acids.

55. The method of claim 54 wherein said single stranded nucleic acids are ribonucleic acids.

Sub B7 56. The method of claim 55 wherein said nucleic acids comprise modified nucleic acids.

57. The method of claim 56 wherein said nucleic acids are 2'-amino (2'-NH₂) modified ribonucleic acids.

58. The method of claim 56 wherein said nucleic acids are 2'-F modified ribonucleic acids.

59. A method for treating hKGF-mediated pathological conditions comprising administering a pharmaceutically effective amount of a hKGF nucleic acid ligand.

60. The method of claim 59 wherein said hKGF nucleic acid ligand is identified according to the method of claim 52.

61. The method of claim 60 wherein said ligand is selected from one of the ligands of Tables 16 and 23 (SEQ ID NOS:189-262, 272-304).

62. A method of assaying a test compound for the ability to inhibit hKGF receptor-mediated cell proliferation, the method comprising the steps of:

a) contacting the test compound with a hKGF nucleic acid ligand and a keratinocyte growth factor; and

b) detecting the ability of the test compound to inhibit binding between the hKGF nucleic acid ligand and the keratinocyte growth factor.

63. A method for assaying a test compound for the ability to inhibit the interaction of a growth factor with its plasma membrane bound receptor, said method comprising the steps of:

a) solubilizing cells containing said plasma membrane bound receptor;

b) creating a plasma membrane extract of said cells;

c) reacting said extract with labeled growth factor alone and in the presence of the test compound thereby creating complexes;

d) analyzing said complexes by electrophoresis under native conditions;

e) visualizing said complexes by imaging; and

f) comparing the image of said extract with labeled growth factor alone to the image of said extract in the presence of the test compound to determine whether said test compound inhibited the interaction between said growth factor and its plasma membrane bound receptor.

64. The method of claim 63 wherein said growth factor is hKGF.

65. The method of claim 63 wherein said cells are PC-3 cells.

66. The method of claim 63 wherein said test compound is selected from the group consisting of a small molecule, a peptide, and an antibody.

67. The method of claim 63 wherein said imaging is selected from the group consisting of autoradiography and phosphorimaging.

5 68. A method for assaying cells to determine whether they express a growth factor plasma membrane bound receptor, said method comprising the steps of:

- 10 a) solubilizing said cells;
b) creating a plasma membrane extract of said cells;
c) reacting said plasma membrane extract with a labeled growth factor;
d) analyzing the reaction between said plasma membrane extract with said labeled growth factor by electrophoresis under native conditions;
15 e) comparing the electrophoresis of step d) with electrophoresis of labeled growth factor; and
f) visualizing the results of the electrophoresis to determine whether a complex is formed with altered mobility relative to the mobility of a labeled growth factor alone.

20 69. A purified and isolated non-naturally occurring nucleic acid ligand to hKGF.

70. The purified and isolated non-naturally occurring nucleic acid ligand of claim 69 wherein said nucleic acid is single stranded.

71. The purified and isolated non-naturally occurring nucleic acid ligand of claim 70 wherein said nucleic acid is ribonucleic acid.

25 72. A nucleic acid ligand to hKGF identified according to the method comprising:

- 30 a) contacting a candidate mixture of nucleic acids with hKGF, wherein nucleic acids having an increased affinity to hKGF relative to the candidate mixture may be partitioned from the remainder of the candidate mixture; and

b) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture; and

c) amplifying the increased affinity nucleic acids to yield a mixture of nucleic acids enriched for nucleic acid sequences with relatively higher affinity and specificity for binding to hKGF, whereby nucleic acid ligands of hKGF may be identified.

73. The purified and isolated non-naturally occurring ribonucleic acid ligand to hKGF of claim 71 wherein said ligand is selected from the group consisting of the sequences set forth in Tables 16 and 23 (SEQ ID NOS:189-262, 272-304).

74. The purified and isolated non-naturally occurring ribonucleic acid ligand to hKGF of claim 71 wherein said ligand is substantially homologous to and has substantially the same ability to bind hKGF as a ligand selected from the group consisting of the sequences set forth in Tables 16 and 23 (SEQ ID NOS:189-262, 272-304).

75. The purified and isolated non-naturally occurring ribonucleic acid ligand to hKGF of claim 71 wherein said ligand has substantially the same structure and substantially the same ability to bind hKGF as a ligand selected from the group consisting of the sequences set forth in Tables 16 and 23 (SEQ ID NOS:189-262, 272-304).

76. A purified and isolated non-naturally occurring ribonucleic acid ligand to bFGF wherein said ligand has the sequence as shown in SEQ ID NO:267.